Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	111020	(synthetic or variant or modif$ or alter$) near5 (gene$1 or sequence$1 or nucleic acid$1)	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:19
L3	4327	codon near3 (choice$1 or preference$1 or select$)	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:21
L4	4126	1 and 3	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:22
L5	2180	1 same 3	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:27
L6	49028	(transcription factor$1 or splice or promoter$1 or polyadenylat$) near5 (site$1 or sequence$1)	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:26
L7	14346	1 same 6	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:27
L8	1251	5 and 7	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:27
L9	134	1 same 3 same 6	US-PGPUB; USPAT	ADJ	OFF	2004/08/25 16:28

```
  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 10:06:28 ON 25 AUG 2004

=> fil .bec
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 10:06:48 ON 25 AUG 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s (synthetic or variant or modif? or alter?)(5a)(gene/q or nucleic acid#)
FILE 'MEDLINE'
        121539 SYNTHETIC
         58643 VARIANT
        359543 MODIF?
        631953 ALTER?
        170110 NUCLEIC
       1478431 ACID#
        169729 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L1       40974 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
               ACID#)

FILE 'SCISEARCH'
        150092 SYNTHETIC
         62205 VARIANT
        472532 MODIF?
        621439 ALTER?
         31869 NUCLEIC
       1174612 ACID#
         31406 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L2       40575 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
               ACID#)

FILE 'LIFESCI'
         38384 SYNTHETIC
         17702 VARIANT
         91970 MODIF?
        171688 ALTER?
         12540 "NUCLEIC"
        311433 ACID#
         12381 NUCLEIC ACID#
                 ("NUCLEIC"(W)ACID#)
L3       21080 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
               ACID#)

FILE 'BIOTECHDS'
         12453 SYNTHETIC
          8338 VARIANT
         31626 MODIF?
         24266 ALTER?
         37631 NUCLEIC
        127373 ACID#
         37553 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L4       13943 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
               ACID#)
```

```
FILE 'BIOSIS'
        191612 SYNTHETIC
         59943 VARIANT
        361830 MODIF?
        640412 ALTER?
         49238 NUCLEIC
       1306370 ACID#
         48659 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L5       46345 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

FILE 'EMBASE'
        103573 SYNTHETIC
         54345 VARIANT
        322451 MODIF?
        593897 ALTER?
         32902 "NUCLEIC"
       1285302 ACID#
         32621 NUCLEIC ACID#
                 ("NUCLEIC"(W)ACID#)
L6       37544 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

FILE 'HCAPLUS'
        542134 SYNTHETIC
         54542 VARIANT
        875783 MODIF?
        800345 ALTER?
        159274 NUCLEIC
       4330304 ACID#
        158327 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L7       66631 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

FILE 'NTIS'
         18688 SYNTHETIC
          2516 VARIANT
         96083 MODIF?
         89855 ALTER?
          1798 NUCLEIC
         54223 ACID#
          1782 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L8         931 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

FILE 'ESBIOBASE'
         37324 SYNTHETIC
         22490 VARIANT
        134896 MODIF?
        218291 ALTER?
         23250 NUCLEIC
        340126 ACID#
         23138 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L9       22511 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

FILE 'BIOTECHNO'
         41250 SYNTHETIC
         25068 VARIANT
         86734 MODIF?
```

```
        148127 ALTER?
         19939 NUCLEIC
        371908 ACID#
         19837 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L10      27490 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

FILE 'WPIDS'
        209332 SYNTHETIC
         17461 VARIANT
        255063 MODIF?
        415925 ALTER?
         49792 NUCLEIC
        897346 ACID#
         49543 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L11      16597 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

TOTAL FOR ALL FILES
L12     334621 (SYNTHETIC OR VARIANT OR MODIF? OR ALTER?)(5A)(GENE/Q OR NUCLEIC
                ACID#)

=> s codon(3a)(choice# or preference# or select?)
FILE 'MEDLINE'
         33513 CODON
        120064 CHOICE#
         43909 PREFERENCE#
        629127 SELECT?
L13        429 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'SCISEARCH'
         23775 CODON
        114362 CHOICE#
         58296 PREFERENCE#
        798493 SELECT?
L14        421 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'LIFESCI'
         13885 CODON
         19544 CHOICE#
         27603 PREFERENCE#
        202991 SELECT?
L15        311 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'BIOTECHDS'
          4791 CODON
          1425 CHOICE#
           802 PREFERENCE#
         59459 SELECT?
L16        104 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'BIOSIS'
         27810 CODON
         74411 CHOICE#
         59362 PREFERENCE#
        682423 SELECT?
L17        468 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'EMBASE'
         26626 CODON
        105876 CHOICE#
         37145 PREFERENCE#
```

```
        576300 SELECT?
L18        368 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'HCAPLUS'
         32831 CODON
         79749 CHOICE#
         40260 PREFERENCE#
       1102568 SELECT?
L19        649 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'NTIS'
            90 CODON
         19166 CHOICE#
          4813 PREFERENCE#
        162925 SELECT?
L20           2 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'ESBIOBASE'
         13569 CODON
         30746 CHOICE#
         18380 PREFERENCE#
        245235 SELECT?
L21        236 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'BIOTECHNO'
         21971 CODON
          8409 CHOICE#
          7785 PREFERENCE#
        148138 SELECT?
L22        314 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

FILE 'WPIDS'
          2513 CODON
         26192 CHOICE#
          6230 PREFERENCE#
        987036 SELECT?
L23          73 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

TOTAL FOR ALL FILES
L24        3375 CODON(3A)(CHOICE# OR PREFERENCE# OR SELECT?)

=> s l12 and l24
FILE 'MEDLINE'
L25          36 L1 AND L13

FILE 'SCISEARCH'
L26          23 L2 AND L14

FILE 'LIFESCI'
L27          18 L3 AND L15

FILE 'BIOTECHDS'
L28          31 L4 AND L16

FILE 'BIOSIS'
L29          31 L5 AND L17

FILE 'EMBASE'
L30          24 L6 AND L18

FILE 'HCAPLUS'
L31          73 L7 AND L19

FILE 'NTIS'
```

```
L32           0 L8 AND L20

FILE 'ESBIOBASE'
L33          15 L9 AND L21

FILE 'BIOTECHNO'
L34          20 L10 AND L22

FILE 'WPIDS'
L35          22 L11 AND L23

TOTAL FOR ALL FILES
L36         293 L12 AND L24

=> s l36 not 2001-2004/py
FILE 'MEDLINE'
       1967013 2001-2004/PY
L37          30 L25 NOT 2001-2004/PY

FILE 'SCISEARCH'
       3631421 2001-2004/PY
L38          19 L26 NOT 2001-2004/PY

FILE 'LIFESCI'
        359020 2001-2004/PY
L39          16 L27 NOT 2001-2004/PY

FILE 'BIOTECHDS'
         76520 2001-2004/PY
L40          14 L28 NOT 2001-2004/PY

FILE 'BIOSIS'
       1871918 2001-2004/PY
L41          28 L29 NOT 2001-2004/PY

FILE 'EMBASE'
       1683780 2001-2004/PY
L42          21 L30 NOT 2001-2004/PY

FILE 'HCAPLUS'
       3686779 2001-2004/PY
L43          48 L31 NOT 2001-2004/PY

FILE 'NTIS'
         52701 2001-2004/PY
L44           0 L32 NOT 2001-2004/PY

FILE 'ESBIOBASE'
       1050648 2001-2004/PY
L45          12 L33 NOT 2001-2004/PY

FILE 'BIOTECHNO'
        368875 2001-2004/PY
L46          19 L34 NOT 2001-2004/PY

FILE 'WPIDS'
       3446783 2001-2004/PY
L47           3 L35 NOT 2001-2004/PY

TOTAL FOR ALL FILES
L48         210 L36 NOT 2001-2004/PY

=> s (transcription factor# or splice or poly(w)'a' or polyadenylat? or
promoter)(5a)(site# or sequence#)(15a)(reduc? or lower? or decreas?)
```

```
FILE 'MEDLINE'
       226841 TRANSCRIPTION
      2158523 FACTOR#
        91045 TRANSCRIPTION FACTOR#
                (TRANSCRIPTION(W)FACTOR#)
        12312 SPLICE
        54177 POLY
      7807314 'A'
         6670 POLYADENYLAT?
       102361 PROMOTER
       657437 SITE#
       706631 SEQUENCE#
      1129262 REDUC?
       634492 LOWER?
       908489 DECREAS?
L49       983 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
              OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
              DECREAS?)

FILE 'SCISEARCH'
       180008 TRANSCRIPTION
      1264388 FACTOR#
        68513 TRANSCRIPTION FACTOR#
                (TRANSCRIPTION(W)FACTOR#)
        13470 SPLICE
       153612 POLY
      9624103 'A'
         5237 POLYADENYLAT?
       102707 PROMOTER
       689078 SITE#
       566283 SEQUENCE#
      1267124 REDUC?
       712992 LOWER?
       890733 DECREAS?
L50      1233 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
              OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
              DECREAS?)

FILE 'LIFESCI'
        95466 "TRANSCRIPTION"
       289485 FACTOR#
        33534 TRANSCRIPTION FACTOR#
                ("TRANSCRIPTION"(W)FACTOR#)
         6312 SPLICE
        17183 POLY
      2030127 'A'
         4136 POLYADENYLAT?
        56650 PROMOTER
       258037 SITE#
       259735 SEQUENCE#
       282558 REDUC?
       138702 LOWER?
       216684 DECREAS?
L51       905 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
              OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
              DECREAS?)

FILE 'BIOTECHDS'
        14230 TRANSCRIPTION
        34606 FACTOR#
         1793 TRANSCRIPTION FACTOR#
                (TRANSCRIPTION(W)FACTOR#)
         1206 SPLICE
         6358 POLY
```

```
        323006 'A'
          1530 POLYADENYLAT?
         30245 PROMOTER
         33811 SITE#
         98190 SEQUENCE#
         44846 REDUC?
         16363 LOWER?
         21542 DECREAS?
L52        170 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
               OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
               DECREAS?)

FILE 'BIOSIS'
        205991 TRANSCRIPTION
       1207743 FACTOR#
         62046 TRANSCRIPTION FACTOR#
                 (TRANSCRIPTION(W)FACTOR#)
         13250 SPLICE
        139273 POLY
       7719073 'A'
          7527 POLYADENYLAT?
        111949 PROMOTER
        677711 SITE#
        519326 SEQUENCE#
       1182682 REDUC?
        703923 LOWER?
       1026285 DECREAS?
L53        994 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
               OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
               DECREAS?)

FILE 'EMBASE'
        210245 "TRANSCRIPTION"
       1108877 FACTOR#
         60798 TRANSCRIPTION FACTOR#
                 ("TRANSCRIPTION"(W)FACTOR#)
         10885 SPLICE
         47033 POLY
       6772410 'A'
          6883 POLYADENYLAT?
         87509 PROMOTER
        544809 SITE#
        495365 SEQUENCE#
       1065611 REDUC?
        588668 LOWER?
        854645 DECREAS?
L54       1303 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
               OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
               DECREAS?)

FILE 'HCAPLUS'
        256737 TRANSCRIPTION
       1360433 FACTOR#
         119085 TRANSCRIPTION FACTOR#
                 (TRANSCRIPTION(W)FACTOR#)
         15285 SPLICE
        615264 POLY
      18101164 'A'
         10741 POLYADENYLAT?
        150102 PROMOTER
        852424 SITE#
        724307 SEQUENCE#
       1851950 REDUC?
        819040 REDN
```

```
        2302369 REDUC?
                  (REDUC? OR REDN)
        1289399 LOWER?
        2093611 DECREAS?
L55        1717 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
                OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
                DECREAS?)

FILE 'NTIS'
           1687 TRANSCRIPTION
         146390 FACTOR#
            372 TRANSCRIPTION FACTOR#
                  (TRANSCRIPTION(W)FACTOR#)
            460 SPLICE
           5560 POLY
        1649160 'A'
             10 POLYADENYLAT?
            921 PROMOTER
         121027 SITE#
          28227 SEQUENCE#
         177425 REDUC?
          66601 LOWER?
          51141 DECREAS?
L56            0 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
                OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
                DECREAS?)

FILE 'ESBIOBASE'
         103054 TRANSCRIPTION
         399685 FACTOR#
          45149 TRANSCRIPTION FACTOR#
                  (TRANSCRIPTION(W)FACTOR#)
           7894 SPLICE
          15825 POLY
        2133392 'A'
           2581 POLYADENYLAT?
          54889 PROMOTER
         421651 SITE#
         227247 SEQUENCE#
         390336 REDUC?
         213986 LOWER?
         310721 DECREAS?
L57         942 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
                OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
                DECREAS?)

FILE 'BIOTECHNO'
         160885 TRANSCRIPTION
         296524 FACTOR#
          41412 TRANSCRIPTION FACTOR#
                  (TRANSCRIPTION(W)FACTOR#)
           8894 SPLICE
          21682 POLY
        1454372 'A'
           5860 POLYADENYLAT?
          72959 PROMOTER
         222731 SITE#
         375038 SEQUENCE#
         232937 REDUC?
         106436 LOWER?
         171676 DECREAS?
L58         1101 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
                OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
                DECREAS?)
```

```
FILE 'WPIDS'
         12170 TRANSCRIPTION
        148873 FACTOR#
          1897 TRANSCRIPTION FACTOR#
                 (TRANSCRIPTION(W)FACTOR#)
          9528 SPLICE
        155046 POLY
       1692882 'A'
           863 POLYADENYLAT?
         30580 PROMOTER
        115016 SITE#
        233214 SEQUENCE#
       1984326 REDUC?
         61106 REDN
       2009742 REDUC?
                 (REDUC? OR REDN)
       1139302 LOWER?
        204740 DECREAS?
L59        131 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W)'A' OR POLYADENYLAT?
               OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
               DECREAS?)

TOTAL FOR ALL FILES
L60       9479 (TRANSCRIPTION FACTOR# OR SPLICE OR POLY(W) 'A' OR POLYADENYLAT?
                OR PROMOTER)(5A)(SITE# OR SEQUENCE#)(15A)(REDUC? OR LOWER? OR
               DECREAS?)

=> s l12 and l60
FILE 'MEDLINE'
L61          72 L1 AND L49

FILE 'SCISEARCH'
L62          67 L2 AND L50

FILE 'LIFESCI'
L63          52 L3 AND L51

FILE 'BIOTECHDS'
L64          38 L4 AND L52

FILE 'BIOSIS'
L65          70 L5 AND L53

FILE 'EMBASE'
L66          79 L6 AND L54

FILE 'HCAPLUS'
L67         158 L7 AND L55

FILE 'NTIS'
L68           0 L8 AND L56

FILE 'ESBIOBASE'
L69          50 L9 AND L57

FILE 'BIOTECHNO'
L70          67 L10 AND L58

FILE 'WPIDS'
L71          44 L11 AND L59

TOTAL FOR ALL FILES
L72         697 L12 AND L60
```

```
=> s l12(15a)l60
FILE 'MEDLINE'
L73          14 L1 (15A)L49

FILE 'SCISEARCH'
L74          15 L2 (15A)L50

FILE 'LIFESCI'
L75          12 L3 (15A)L51

FILE 'BIOTECHDS'
L76           7 L4 (15A)L52

FILE 'BIOSIS'
L77          11 L5 (15A)L53

FILE 'EMBASE'
L78          21 L6 (15A)L54

FILE 'HCAPLUS'
L79          59 L7 (15A)L55

FILE 'NTIS'
L80           0 L8 (15A)L56

FILE 'ESBIOBASE'
L81          12 L9 (15A)L57

FILE 'BIOTECHNO'
L82          14 L10(15A)L58

FILE 'WPIDS'
L83          15 L11(15A)L59

TOTAL FOR ALL FILES
L84         180 L12(15A) L60

=> s l24 and 160
FILE 'MEDLINE'
L85           0 L13 AND L49

FILE 'SCISEARCH'
L86           0 L14 AND L50

FILE 'LIFESCI'
L87           0 L15 AND L51

FILE 'BIOTECHDS'
L88           0 L16 AND L52

FILE 'BIOSIS'
L89           0 L17 AND L53

FILE 'EMBASE'
L90           0 L18 AND L54

FILE 'HCAPLUS'
L91           1 L19 AND L55

FILE 'NTIS'
L92           0 L20 AND L56

FILE 'ESBIOBASE'
```

```
L93             0 L21 AND L57

FILE 'BIOTECHNO'
L94             0 L22 AND L58

FILE 'WPIDS'
L95             0 L23 AND L59

TOTAL FOR ALL FILES
L96             1 L24 AND L60

=> s (l84 or l96) not 2001-2004/py
FILE 'MEDLINE'
       1967013 2001-2004/PY
L97           11 (L73 OR L85) NOT 2001-2004/PY

FILE 'SCISEARCH'
       3631421 2001-2004/PY
L98           11 (L74 OR L86) NOT 2001-2004/PY

FILE 'LIFESCI'
        359020 2001-2004/PY
L99           12 (L75 OR L87) NOT 2001-2004/PY

FILE 'BIOTECHDS'
         76520 2001-2004/PY
L100           4 (L76 OR L88) NOT 2001-2004/PY

FILE 'BIOSIS'
       1871918 2001-2004/PY
L101           9 (L77 OR L89) NOT 2001-2004/PY

FILE 'EMBASE'
       1683780 2001-2004/PY
L102          16 (L78 OR L90) NOT 2001-2004/PY

FILE 'HCAPLUS'
       3686779 2001-2004/PY
L103          21 (L79 OR L91) NOT 2001-2004/PY

FILE 'NTIS'
         52701 2001-2004/PY
L104           0 (L80 OR L92) NOT 2001-2004/PY

FILE 'ESBIOBASE'
       1050648 2001-2004/PY
L105           8 (L81 OR L93) NOT 2001-2004/PY

FILE 'BIOTECHNO'
        368875 2001-2004/PY
L106          14 (L82 OR L94) NOT 2001-2004/PY

FILE 'WPIDS'
       3446783 2001-2004/PY
L107           3 (L83 OR L95) NOT 2001-2004/PY

TOTAL FOR ALL FILES
L108         109 (L84 OR L96) NOT 2001-2004/PY

=> dup rem l48,l108
PROCESSING COMPLETED FOR L48
PROCESSING COMPLETED FOR L108
L109         105 DUP REM L48 L108 (214 DUPLICATES REMOVED)
```

=> d tot

L109 ANSWER 1 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Hygromycin-tolerant **gene** with CTG codon **modified** into leucine **codon**, applicable as **selection** marker in yeast of Candida genus providing transformants for efficient production of e.g. dicarboxylic acid;
plasmid pUCARS-HGM-mediated gene transfer and expression in Candida tropicalis
AU Tanaka A; Ueda M; Hara A; Misawa A
AN 2001-04352 BIOTECHDS
PI WO 2000075307 14 Dec 2000

L109 ANSWER 2 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Constructing **synthetic** polynucleotide for targeting expression of **gene** to particular cells or tissues, involves subsituting one or more codons or parent polynucleotide encoding protein with a synonymous codon;
plasmid pAOV2-mediated gene transfer and expression in Escherichia coli or transgenic plant using Agrobacterium sp. for gene targeting
AU Zhou J; Frazer I H; Botella Mesa J R
AN 2000-12546 BIOTECHDS
PI WO 2000042190 20 Jul 2000

L109 ANSWER 3 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Genotype analysis method, defined as SOMA (short oligonucleotide mass analysis), of short, defined amplication products using electro-spray ionization mass spectrometry, useful for analyzing the genotype of living organisms;
for human genotyping and polymorphism detection using DNA primer
AU Laken S J; Vogelstein B; Kinzler K W; Groopman J D; Jackson P E; Friesen M D
AN 2000-11281 BIOTECHDS
PI WO 2000031300 2 Jun 2000

L109 ANSWER 4 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI **Modified synthetic** DNA **sequences** comprise **modification** of the truncated cry9Aa **gene** of Bacillus thuringiensis for improved insect control in plants;
transgenic plant construction with improved disease-resistance
AU Kuvshinov V; Kanerva A; Koivu K; Pehu E
AN 2000-06780 BIOTECHDS
PI WO 2000011025 2 Mar 2000

L109 ANSWER 5 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Recombinant bioadhesive protein analogs comprising hydroxyproline
SO PCT Int. Appl., 52 pp.
CODEN: PIXXD2
IN Paolella, David N.; Gruskin, Elliott A.; Buechter, Douglas D.
AN 2000:191212 HCAPLUS
DN 132:232726

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000015789	A1	20000323	WO 1999-US20463	19990907
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
AU 9959100	A1	20000403	AU 1999-59100	19990907

L109 ANSWER 6 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
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CODEN: BBACAQ; ISSN: 0006-3002
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DN 133:219415

L109 ANSWER 7 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
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CODEN: NDZKEJ; ISSN: 1000-1638
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CODEN: FCFUEO; ISSN: 0915-8502
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L109 ANSWER 10 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Molecular biology of spider silk;
spider silk fibroin protein engineering and expression in silkworm transgenic insect; a review
SO Rev.Mol.Biotechnol.; (2000) 74, 2, 85-93
CODEN: RMBIFZ ISSN: 1389-0352
AU Winkler S; *Kaplan D
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L109 ANSWER 11 OF 105 MEDLINE on STN DUPLICATE 3
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Journal code: 7705721. ISSN: 0378-1097.
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L109 ANSWER 12 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI An optimized DNA molecule encoding a protein that degrades the herbicide dalapon;
wheat transgenic plant exhibiting herbicide resistance against dalapon
AU Gressel J; Galun E; Zhang J
AN 1999-10548 BIOTECHDS
PI WO 9927116 3 Jun 1999

L109 ANSWER 13 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
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recombinant ferric iron-reductase used to increase the iron content of

rice and tobacco transgenic plant particularly to increase the nutritional content
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L109 ANSWER 14 OF 105 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
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PI WO 9913089 A1 19990318 (199921)* EN 34 C12N015-56
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
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L109 ANSWER 15 OF 105 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
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L109 ANSWER 17 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
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Journal code: 0411011. ISSN: 0305-1048.
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W: AU, CA, IL, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
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L109 ANSWER 59 OF 105 MEDLINE on STN DUPLICATE 28
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L109 ANSWER 67 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
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SO Protein Engineering (1992), 5(8), 821-5 CODEN: PRENE9; ISSN: 0269-2139
AU Libertini, Giacinto; Di Donato, Alberto
AN 1993:117657 HCAPLUS
DN 118:117657

L109 ANSWER 68 OF 105 MEDLINE on STN DUPLICATE 33
TI High-level production of active HIV-1 protease in Escherichia coli.
SO Gene, (1992 Dec 15) 122 (2) 263-9. Journal code: 7706761. ISSN: 0378-1119.
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AN 93138395 MEDLINE

L109 ANSWER 69 OF 105 MEDLINE on STN DUPLICATE 34
TI Translation of the first gene of the Escherichia coli unc operon. **Selection** of the start **codon** and control of initiation efficiency.
SO Journal of biological chemistry, (1991 Nov 5) 266 (31) 21090-8. Journal code: 2985121R. ISSN: 0021-9258.

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AN 92041981 MEDLINE

L109 ANSWER 70 OF 105 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
TI TRANSLATION OF THE 1ST GENE OF THE ESCHERICHIA-COLI UNC OPERON - **SELECTION** OF THE START **CODON** AND CONTROL OF INITIATION EFFICIENCY
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1991) Vol. 266, No. 31, pp. 21090-21098.
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AN 91:606651 SCISEARCH

L109 ANSWER 71 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI mRNA leader length and initiation codon context determine **alternative** AUG selection for the yeast **gene** MOD5
SO Proceedings of the National Academy of Sciences of the United States of America (1991), 88(21), 9789-93
CODEN: PNASA6; ISSN: 0027-8424
AU Slusher, Leslie B.; Gillman, Edwin C.; Martin, Nancy C.; Hopper, Anita K.
AN 1992:1612 HCAPLUS
DN 116:1612

L109 ANSWER 72 OF 105 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 35
TI UTILIZATION OF DNA RECOMBINATION FOR THE 2-STEP REPLACEMENT OF GROWTH-FACTOR SEQUENCES IN THE VACCINIA VIRUS GENOME
SO JOURNAL OF VIROLOGY, (1991) Vol. 65, No. 9, pp. 4609-4618.
AU SPYROPOULOS D D (Reprint); STALLARD V; ROBERTS B E; COHEN L K
AN 91:465709 SCISEARCH

L109 ANSWER 73 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Codon utilization in the pathogenic yeast, Candida albicans
SO Nucleic Acids Research (1991), 19(15), 4298
CODEN: NARHAD; ISSN: 0305-1048
AU Brown, Alistair J. P.; Bertram, Gwyneth; Feldmann, Pascale J. F.; Peggie, Mark W.; Swoboda, Rolf K.
AN 1991:600069 HCAPLUS
DN 115:200069

L109 ANSWER 74 OF 105 MEDLINE on STN DUPLICATE 36
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SO BioTechniques, (1991 Jun) 10 (6) 782-4.
Journal code: 8306785. ISSN: 0736-6205.
AU Tamura T; Holbrook S R; Kim S H
AN 91345888 MEDLINE

L109 ANSWER 75 OF 105 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
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SO BIOTECHNIQUES, (1991) Vol. 10, No. 6, pp. 782-784.
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AN 91:340084 SCISEARCH

L109 ANSWER 76 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Activity of promoter mutants of the yeast ribosomal RNA gene with and without the enhancer
SO Yeast (1991), 7(7), 679-89
CODEN: YESTE3; ISSN: 0749-503X
AU Butlin, Mike; Quincey, Roger
AN 1991:649452 HCAPLUS
DN 115:249452

L109 ANSWER 77 OF 105 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 37

TI **ALTERATION** IN THE -10 **SEQUENCE** OF THE A2 **PROMOTER** OF BACTERIOPHAGE T7 **REDUCES** THE RATE OF TRANSCRIPTION INITIATION
SO CURRENT SCIENCE, (1991) Vol. 60, No. 9-10, pp. 594-596.
AU KUMAR K P (Reprint); GOPAL V; CHATTERJI D
AN 91:478589 SCISEARCH

L109 ANSWER 78 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Production of bovine insulin-like growth factor 2 (bIGF2) in Escherichia coli
SO Gene (1991), 101(2), 291-5
CODEN: GENED6; ISSN: 0378-1119
AU Easton, Alan M.; Giersè, James K.; Seetharam, Ramnath; Klein, Barbara K.; Kotts, Claire E.
AN 1992:52779 HCAPLUS
DN 116:52779

L109 ANSWER 79 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Optimization of the signal-sequence cleavage site for secretion from Bacillus subtilis of a 34-amino acid fragment of human parathyroid hormone
SO Gene (1991), 102(2), 277-82
CODEN: GENED6; ISSN: 0378-1119
AU Saunders, Charles W.; Pedroni, Julia A.; Monahan, Paula M.
AN 1992:1654 HCAPLUS
DN 116:1654

L109 ANSWER 80 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Strategy for constructing **synthetic genes** for efficient expression in bacteria;
e.g. human interleukin-2 artificial gene construction and expression in Escherichia coli (conference paper)
SO Biol.Recombinant Microorg.Anim.Cells; (1991) Oholo 34 Meet., 83-89
AU Leitner M; Cohen S; Lion M; Flashner Y; Katzir N; Grosfeld H
AN 1992-06041 BIOTECHDS

L109 ANSWER 81 OF 105 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 38
TI Identification and characterization of two upstream elements that regulate adrenocortical expression of steroid 11β-hydroxylase.
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ISSN: 0888-8809 CODEN: MOENEN
AU Bogerd A.M.; Franklin A.; Rice D.A.; Schimmer B.P.; Parker K.L.
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L109 ANSWER 82 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Chemo-enzymatic synthesis of optically pure l-leucovorin, an augmentor of 5-fluorouracil cytotoxicity against cancer;
Escherichia coli dihydrofolate-reductase and Gluconobacter scleroideus glucose-dehydrogenase; NADPH coenzyme regeneration; potential mamma and colon tumor therapy
SO Biochem.Biophys.Res.Commun.; (1990) 171, 2, 684-89
CODEN: BBRCA9
AU Uwajima T; Oshiro T; Eguchi T; Kuge Y; Horiguchi A; Igarashi A
AN 1990-13492 BIOTECHDS

L109 ANSWER 83 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Gene synthesis of substance P N-terminal (1-5)
SO Zeitschrift fuer Chemie (1990), 30(7), 253
CODEN: ZECEAL; ISSN: 0044-2402
AU Meister, Walter Vesely; Birch-Hirschfeld, Eckhard; Reinert, Hilmar; Hoffmann, Siegfried
AN 1990:606189 HCAPLUS
DN 113:206189

L109 ANSWER 84 OF 105 MEDLINE on STN DUPLICATE 39
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SO Biochimica et biophysica acta, (1990 Apr 6) 1048 (2-3) 156-64.
Journal code: 0217513. ISSN: 0006-3002.
AU Chambers J; Raymond G J; Kim D; Raymond K C; Nelson C; Clark S; Johnson J D
AN 90212645 MEDLINE

L109 ANSWER 85 OF 105 MEDLINE on STN
TI Mutations in the structural genes for eukaryotic initiation factors 2 alpha and 2 beta of Saccharomyces cerevisiae disrupt translational control of GCN4 mRNA.
SO Proceedings of the National Academy of Sciences of the United States of America, (1989 Oct) 86 (19) 7515-9.
Journal code: 7505876. ISSN: 0027-8424.
AU Williams N P; Hinnebusch A G; Donahue T F
AN 90017508 MEDLINE

L109 ANSWER 86 OF 105 MEDLINE on STN DUPLICATE 40
TI Codon contexts from weakly expressed genes reduce expression in vivo.
SO Journal of molecular biology, (1989 Oct 5) 209 (3) 359-78.
Journal code: 2985088R. ISSN: 0022-2836.
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AN 90064499 MEDLINE

L109 ANSWER 87 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Construction of linker-scanning mutations using a kanamycin-resistance cassette with multiple symmetric restriction sites;
linker-scanning site-directed mutagenesis method using a DNA cassette in the plasmid pKlink vector, e.g. mouse dihydrofolate-**reductase gene promoter** DNA **sequence variant** construction
SO Gene; (1989) 84, 1, 159-64
CODEN: GENED6
AU Smith M L; *Crouse G F
AN 1990-05453 BIOTECHDS

L109 ANSWER 88 OF 105 MEDLINE on STN DUPLICATE 41
TI Definition of cis-acting elements regulating expression of the Drosophila melanogaster ninaE opsin gene by oligonucleotide-directed mutagenesis.
SO Genetics, (1989 Jan) 121 (1) 77-87.
Journal code: 0374636. ISSN: 0016-6731.
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AN 89137954 MEDLINE

L109 ANSWER 89 OF 105 MEDLINE on STN DUPLICATE 42
TI Protein engineering of antibody binding sites: recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in Escherichia coli.
SO Proceedings of the National Academy of Sciences of the United States of America, (1988 Aug) 85 (16) 5879-83.
Journal code: 7505876. ISSN: 0027-8424.
AU Huston J S; Levinson D; Mudgett-Hunter M; Tai M S; Novotny J; Margolies M N; Ridge R J; Bruccoleri R E; Haber E; Crea R; +
AN 88320347 MEDLINE

L109 ANSWER 90 OF 105 LIFESCI COPYRIGHT 2004 CSA on STN
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SO NUCLEIC ACIDS RES., (1988) vol. 16, no. 8, pp. 3391-3404.
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AN 88:64284 LIFESCI

L109 ANSWER 91 OF 105 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 43
TI cis-Acting intron mutations that affect the efficiency of avian retroviral RNA splicing: Implication for mechanisms of control.
SO Journal of Virology, (1988) 62/8 (2686-2695).
ISSN: 0022-538X CODEN: JOVIAM
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AN 88193862 EMBASE

L109 ANSWER 92 OF 105 MEDLINE on STN DUPLICATE 44
TI Chemical synthesis and cloning of secretin gene.
SO Scientia Sinica. Series B, Chemical, biological, agricultural, medical & earth sciences / Chung-kuo k'o hsueh yuan, chu pan, (1988 Jun) 31 (6) 687-94.
Journal code: 8209875. ISSN: 0253-5823.
AU Qian S W; Chen C Q; Li Z P
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L109 ANSWER 93 OF 105 MEDLINE on STN DUPLICATE 45
TI Sequence specificity of mutations induced by benzo[a]pyrene-7,8-diol-9,10-epoxide at endogenous aprt gene in CHO cells.
SO Somatic cell and molecular genetics, (1988 Jul) 14 (4) 393-400.
Journal code: 8403568. ISSN: 0740-7750.
AU Mazur M; Glickman B W
AN 88290826 MEDLINE

L109 ANSWER 94 OF 105 MEDLINE on STN DUPLICATE 46
TI Influence of the codon following the AUG initiation codon on the expression of a **modified** lacZ **gene** in Escherichia coli.
SO EMBO journal, (1987 Aug) 6 (8) 2489-92.
Journal code: 8208664. ISSN: 0261-4189.
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AN 88029345 MEDLINE

L109 ANSWER 95 OF 105 MEDLINE on STN
TI Expression of mouse dihydrofolate reductase gene confers methotrexate resistance in transgenic petunia plants.
SO Somatic cell and molecular genetics, (1987 Jan) 13 (1) 67-76.
Journal code: 8403568. ISSN: 0740-7750.
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AN 87120552 MEDLINE

L109 ANSWER 96 OF 105 MEDLINE on STN DUPLICATE 47
TI Cloning of a portion of the chromosomal gene for human erythrocyte alpha-spectrin by using a **synthetic gene** fragment.
SO Proceedings of the National Academy of Sciences of the United States of America, (1986 Apr) 83 (8) 2397-401.
Journal code: 7505876. ISSN: 0027-8424.
AU Linnenbach A J; Speicher D W; Marchesi V T; Forget B G
AN 86205962 MEDLINE

L109 ANSWER 97 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Evolution of the growth hormone gene family
SO American Zoologist (1986), 26(4), 939-49
CODEN: AMZOAF; ISSN: 0003-1569
AU Slater, Emily P.; Baxter, John D.; Eberhardt, Norman L.
AN 1987:61857 HCAPLUS
DN 106:61857

L109 ANSWER 98 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Production of human alpha consensus interferon in recombinant Escherichia

coli;
high level expression system regulation and controlled feeding schedule application
SO Chem.Eng.Commun.; (1986) 45, 1-6, 229-40
CODEN: CEGCAK
AU Fieschko J; Ritch T
AN 1987-06777 BIOTECHDS

L109 ANSWER 99 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Microbial expression of type I transforming growth factor, its polypeptide analogs and hybrid EGF/TGF polypeptides
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
IN Banks, Allen R.; Hare, David L.
AN 1985:555264 HCAPLUS
DN 103:155264

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 8502198	A1	19850523	WO 1984-US1747	19841030
W: JP				
JP 61500250	T2	19860220	JP 1984-504146	19841030
EP 150572	A1	19850807	EP 1984-307490	19841031
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				

L109 ANSWER 100 OF 105 MEDLINE on STN DUPLICATE 49
TI Expression of a **synthetic** human growth hormone **gene** in yeast.
SO Gene, (1985) 39 (1) 117-20.
Journal code: 7706761. ISSN: 0378-1119.
AU Tokunaga T; Iwai S; Gomi H; Kodama K; Ohtsuka E; Ikehara M; Chisaka O; Matsubara K
AN 86083187 MEDLINE

L109 ANSWER 101 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI DNA sequences, recombinant DNA molecules and processes for producing bovine growth hormone-like polypeptides in high yield
SO Eur. Pat. Appl., 31 pp.
CODEN: EPXXDW
IN Buell, Gary Nutter
AN 1984:418487 HCAPLUS
DN 101:18487

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 103395	A2	19840321	EP 1983-304574	19830808
EP 103395	A3	19850522		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
CA 1224432	A1	19870721	CA 1983-434118	19830808
ZA 8305880	A	19840425	ZA 1983-5880	19830810
US 4693973	A	19870915	US 1983-522357	19830811
DK 8303752	A	19840218	DK 1983-3752	19830816
NO 8302948	A	19840220	NO 1983-2948	19830816
AU 8318021	A1	19840223	AU 1983-18021	19830816
AU 568597	B2	19880107		
JP 59063197	A2	19840410	JP 1983-148857	19830816
HU 32154	O	19840628	HU 1983-2883	19830816
ES 524971	A1	19850101	ES 1983-524971	19830816
DD 212982	A5	19840829	DD 1983-254030	19830817

L109 ANSWER 102 OF 105 MEDLINE on STN DUPLICATE 50
TI Complete sequence of the cDNA for human alpha 1-antitrypsin and the **gene** for the S **variant.**
SO Biochemistry, (1984 Oct 9) 23 (21) 4828-37.
Journal code: 0370623. ISSN: 0006-2960.
AU Long G L; Chandra T; Woo S L; Davie E W; Kurachi K

AN 85047190 MEDLINE

L109 ANSWER 103 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
TI The manufacture and expression of structural genes
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
IN Stabinsky, Yitzhak
AN 1984:133585 HCAPLUS
DN 100:133585

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8304029	A1	19831124	WO 1983-US563	19830415
	W: JP				
	RW: AT, BE, CH, DE, FR, GB, LU, NL, SE				
	US 4652639	A	19870324	US 1982-375493	19820506
	EP 108787	A1	19840523	EP 1983-901773	19830415
	EP 108787	B1	19900411		
	R: AT, BE, CH, DE, FR, GB, LI, LU, NL, SE				
	JP 59501096	T2	19840628	JP 1983-501807	19830415
	JP 07089934	B4	19951004		
	AT 51873	E	19900415	AT 1983-901773	19830415
	IL 68491	A1	19900726	IL 1983-68491	19830426
	CA 1266628	A1	19900313	CA 1983-427371	19830504

L109 ANSWER 104 OF 105 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Production of genes for Escherichia coli transformation - useful in preparation of
urogastrone polypeptide and analogues.

PI	WO 8304030	A	19831124 (198348)* EN	34
	RW: AT BE CH DE FR GB LU NL SE			
	W: JP			
	EP 108132	A	19840516 (198421)	EN
	R: AT BE CH DE FR GB LI LU NL SE			
	CA 1214739	A	19861202 (198701)	
	US 4764593	A	19880816 (198835)	
	IL 68605	A	19910730 (199133)	
	IT 1212984	B	19891207 (199150)	

IN BANKS, A R

L109 ANSWER 105 OF 105 MEDLINE on STN DUPLICATE 51
TI The leftward promoter of bacteriophage lambda. Structure, biological activity, and influence by adjacent regions.
SO Journal of biological chemistry, (1981 Feb 25) 256 (4) 2003-9.
Journal code: 2985121R. ISSN: 0021-9258.
AU Horn G T; Wells R D
AN 81117294 MEDLINE

=>
=> d ab
2,4,7,10-13,19,21,27,30,33,36,41,42,44,46,47,49,54,56,57,66-68,74,78,80,92,96,98,100,103

L109 ANSWER 2 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB Constructing a synthetic polynucleotide which selectively expresses a protein in a target cell from plant, relative to another cell from the plant by **selecting** a first **codon** of parent polynucleotide and replacing it with synonymous codon which has a higher translational efficiency in the target cell than the other cell, is new. Also claimed are: a polynucleotide (762 or 780 bp) constructed by the method; a vector (e.g. plasmid pAOV2); a cell (e.g. Escherichia coli) containing the polynucleotide; a cell containing the vector; a transgenic plant or its parts produced using Agrobacterium sp.-mediated transfer; selectively expressing a protein in a target plant cell; expressing a

protein from a first nucleotide in a target plant cell; and a cell produced by the method. To facilitate selective expression of proteins to a particular target plant cell or tissue and target expression to particular cells or tissues to produce transgenic plants with novel phenotypes e.g. conferring herbicides the resistance in leaves of a plant but not in the roots of the plant. (106pp)

L109 ANSWER 4 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB A **modified synthetic** DNA **sequence** (I) for improved insect control comprising DNA modified from the truncated crystal protein cry9Aa gene of Bacillus thuringiensis ssp. galleria are claimed. The encoded crystal protein has a disclosed protein sequence (Ia) of 624 amino acid residues or has a **sequence alteration**, but the same activity as the active N-terminal domain of the Cry9Aa protein. Also claimed are: a DNA construct for cloning and/or transforming prokaryotic or eukaryotic organisms comprising (I); a prokaryotic or eukaryotic host comprising (I); a method for preparing (I) involving selecting a DNA sequence encoding (Ia) and the unique properties of the Cry9Aa protein and differing from other CryI proteins, providing **synthetic** DNA **sequences** encoding (Ia), which is encoded by the truncated DNA sequence of 1,989 bp obtainable from the native cry9Aa gene having a 3,837 bp sequence by trypsin (EC-3.4.21.4) cleavage, and improving translation by changing the **codon preference**; and a method for providing higher plants with insect resistance involving incorporating (I) into a DNA construct and incorporating the construct into a plant to give a transgenic plant. (90pp)

L109 ANSWER 7 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB Codon usage varies throughout the animal and plant kingdoms. The flounder antifreeze peptide (AFP) is Ala-rich (60%) and the Ala codons of the fish cDNA reveal a strong bias toward GCC (23/38). We have taken the opportunity to modify the **codon selection** for all the amino acids to be consistent with normal usage in plant genes. The pre-sequence of AFP was replaced by the pre- sequence of sweet potato Sporamin A (EMBL X13 509), and the pro sequence was not included. The **modified genes** coding 2,4,8 tandem mature AFP were constructed and confirmed by DNA sequencing. Using QIA express system. expression of DHFR-mature AFP constructs in E. coli suggests the **modified synthetic** AFP **gene** could be work in plants.

L109 ANSWER 10 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB Molecular biology of spider silk was studied with respect to silk processing, genetic engineering and potential application. In vitro production of silk from excised major ampullate gland was demonstrated. A total of 2.3 kb was sequenced. cDNA clones of greater than 2.3 kb for the major ampullate silk gene was unstable, gene **codon preferences** and incompatibility with prokaryote hosts such as Escherichia coli was speculated as a problem due to mismatched tRNA availability. The use of **synthetic genes**, constructed from small size oligonucleotide repeats allowed control over primary sequence and final protein size. Expression levels from the **synthetic genes** were low and generally represented up to 5% of the total protein in the cell. Yield were low and generally in the 1-10 mg/l range, depending on the size of the protein. Additional research directions for bioengineering spider silk proteins e.g. fibroin include transgenic expression in the silkworm as well as other organisms as a route to improving yields of the materials in order to explore and extend the potential applications for these materials. (60 ref)

L109 ANSWER 11 OF 105 MEDLINE on STN DUPLICATE 3
AB The catalytic domain of the xynB (xylanase) gene from the thermophilic bacterium Dictyoglomus thermophilum was reconstructed by PCR to match the

codon preference of Trichoderma reesei. The 0.6-kb DNA fragment encoding the enzyme was first amplified by primer extension with a mixture of eight overlapping oligonucleotides, followed by PCR with outside primers containing restriction enzyme sites for directional cloning into Escherichia coli and T. reesei vectors. The **synthetic gene** was expressed in both organisms, producing a clearing halo around transformant colonies in plate assay utilizing an overlay of oat spelts xylan. Effective transcription of xyn B in T. reesei was obtained after changing 20 codons.

L109 ANSWER 12 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB A DNA molecule (I) encoding a dehalogenase that is capable of dalapon herbicide pesticide degradation is claimed, where the DNA has codon usage suitable for small grain species. Also claimed are: a vector which contains (I); an expression cassette containing (I) or a vector, which is suitable for the transformation of plant cells and plants of small grain species and to confer dalapon-resistance to regenerated plants; a transgenic plant of a small grain species containing (I) and that is substantially resistant to dalapon at field use levels; seeds and plants of small grain species which possess, stably integrated in their genome, a foreign DNA such as (I) sufficient to render the small grain species resistant to dalapon at field use levels; a method for rendering a plant of a small grain species resistant to dalapon at field use levels; and a method for protecting plants of a small grain species and destroying weeds in a field using the herbicide dalapon. (I) is Pseudomonas sp. dehal **gene** with its **codon preference altered.** The plants are preferably wheat (Triticum aestivum) and exhibit herbicide resistance. (29pp)

L109 ANSWER 13 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB A means of transforming a higher plant with a foreign gene containing a modified polyadenylation signal sequence, is claimed. Also claimed is a plant transformed in this manner, the seed of that plant, and nucleic acids containing the modified gene sequence. This is used to produce transgenic plants that produce an increased amount of a desired gene, particularly to improve the iron absorption efficiency of crop plants. The **modified gene** preferably encodes a protein involved in nutriment absorption, such as a ferric iron-**reductase** gene derived from yeast. The **polyadenylation sequence** is **modified** by base substitution, based on the codon use ratio of the plant to be transformed, preferably so that the modified codons reduce the same amino acid residues as the original. The foreign gene preferably includes a Kozak initiation sequence upstream of the start codon of the gene. This is particularly used in the production of transgenic tobacco (Nicotiana tabacum) and transgenic rice (Oryza sativa). The vector is specifically vector plasmid pBI121, which is transferred into plant cells using Agrobacterium tumefaciens. (81pp)

L109 ANSWER 19 OF 105 MEDLINE on STN DUPLICATE 4
AB The Plasmodium falciparum malaria parasite is the causative agent of malaria tropica. Merozoites, one of the extracellular developmental stages of this parasite, expose at their surface the merozoite surface protein-1 complex (MSP-1), which results from the proteolytic processing of a 190-200 kDa precursor. MSP-1 is highly immunogenic in humans and numerous studies suggest that this protein is an effective target for a protective immune response. Although its function is unknown, there are indications that it may play a role during invasion of erythrocytes by merozoites. The parasite-derived msp-1 gene, which is approximately 5000 bp long, contains 74% AT. This high AT content has prevented stable cloning of the full-size gene in Escherichia coli and consequently its expression in heterologous systems. Here, we describe the synthesis of a 4917 bp gene encoding MSP-1 from the FCB-1 strain of P. falciparum adjusted for human **codon preferences.** The **synthetic** msp-1 **gene** (55% AT) was cloned, maintained and

expressed in its entirety in E.coli as well as in CHO and HeLa cells. The purified protein is soluble and appears to possess native conformation because it reacts with a panel of mAbs specific for conformational epitopes. The strategy we used for synthesizing the full-length msp-1 gene was toassemble it from DNA fragments encoding all of the major proteolytic fragments normally generated at the parasite's surface. Thus, after subcloning we also obtained each of these MSP-1 processing products as hexahistidine fusion proteins in E.coli and isolated them by affinity chromatography on Ni2+agarose. The availability of defined preparations of MSP-1 and its major processing products open up new possibilities for in-depth studies at the structural and functional level of this important protein, including the exploration of MSP-1-based experimental vaccines.

L109 ANSWER 21 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB The invention provides a compound comprising a nucleic acid encoding an 85 antigen of Mycobacterium, wherein at least one codon of the **nucleic acid** encoding the 85 antigen is **altered** whereby expression of the 85 antigen in an expression system is increased over expression in the same expression system of a nucleic acid having a wild-type codon at the same position as the altered codon. Specifically, the invention provides an isolated nucleic acid encoding a protein of Mycobacterium comprising at least one codon **altered** to increase expression of the **nucleic acid**, wherein the **codon** is **selected** from the group consisting of AGG, AGA, ATA, CGA, CTA, CGG, CTT, CTC, GGG, and GGA and an isolated nucleic acid encoding an 85 antigen of Mycobacterium comprising at least one codon **altered** to increase expression of the **nucleic acid**, wherein the **codon** is **selected** from the group consisting of AGG, AGA, ATA, CTA, CGC, CTT, CTC, GGG, and GGA. Also provided is an improved method of producing a Mycobacterium protein in a host cell comprising **altering** a codon of a **nucleic acid** encoding the Mycobacterium protein so that the **altered** codon of the **nucleic acid** is one preferred by the host and introducing the **nucleic acid** containing the **altered** codon into the host, whereby the host expresses the nucleic acid thereby producing the Mycobacterium protein. When the wild-type antigen 85C gene was expressed in E. coli, the yield was 15-20 mg antigen/L. When five codons in this gene were changed to E. coli-preferred codons, the yield increased to >60 mg/L.

L109 ANSWER 27 OF 105 MEDLINE on STN DUPLICATE 7
AB **Synthetic genes** are very useful in genetic and protein engineering. Here we propose a general method for construction of **synthetic genes.** Short oligonucleotides are joined through ligase chain reaction (LCR) in high stringency conditions to make "unit fragments" which are then fused to form a full-length gene sequence by polymerase chain reaction. The procedure is simple and accurate and does not place constraints on sequence and length. In this report, a recombinant leptin gene was synthesized according to the **codon preference** of Escherichia coli. Besides, a substitution of the only Met at position 54 for Leu and an addition of a Met at the N-terminus were introduced in the **synthetic gene.** The gene was cloned in the pQE-31 expression vector and was expressed in E. coli. A large amount of recombinant leptin containing 6 x His tag was produced and purified by Ni-NTA affinity column. Finally, intact leptin-L54 was released after removing the tag by CNBr cleavage at the Met residue.

L109 ANSWER 30 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB A novel cecropin AD gene was designed and synthesized, in which DNA sequence is based on the amino acid sequence of hybrid peptide cecropin A1-11D12-37. The **codon selection** of the designed gene was carried out according to the coden usage of yeast. The designed gene is 140 bp in length, including the encoded sequence, the start and stop codons, and the restriction sites of BamH I, EcoR I and Sal I at both

ends. The cecropin AD gene was synthesized by twice PCR method and cloned into PCRTM 2.1 vector. It was shown that the DNA **sequence** of the **synthetic** cecropin AD **gene** coincides with that of the designed gene by DNA sequencing.

L109 ANSWER 33 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB A review with 60 refs. of an ongoing process to genetically engineer durable pathogen resistance into poplar and other tree species. The process involved designing, testing, and selecting antimicrobial peptide sequences to use as transgenic plant products. The **synthetic** peptide **sequence** were then encoded into DNA. Gene promoters, translation initiation and termination sequences, **codon preferences**, mRNA secondary structure, peptide stability and peptide targeting were considered when designing gene constructs to test in transgenic plants.

L109 ANSWER 36 OF 105 MEDLINE on STN DUPLICATE 12
AB **Synthetic genes** were designed to encode analogs of the two proteins of Nephila clavipes dragline silk, spidroins 1 and 2. The genes were constructed of tandem repeats of relatively long (more than 300 bp) DNA **sequences** assembled from **synthetic** oligonucleotides, and encoded proteins of high molecular mass (65-163 kDa). Both analogs were produced efficiently in Escherichia coli. The yield and homogeneity of the products of longer genes were limited by premature termination of synthesis, probably as a result of processivity errors in protein synthesis. Average termination rates were determined to be 1 in 1100 codons to 1 in 300 codons, depending on the length and synonymous **codon choices** of the gene. Both analog proteins could be induced to form stable aqueous solutions without denaturants. Circular dichroism spectra of the purified proteins in dilute solution resembled spectra of redissolved natural dragline silk in reflecting a largely disordered structure in water and more ordered structures in mixed solvents with methanol and trifluoroethanol.

L109 ANSWER 41 OF 105 MEDLINE on STN DUPLICATE 15
AB BACKGROUND. The expression of both the env and gag gene products of human immunodeficiency virus type 1 (HIV-1) is known to be limited by cis elements in the viral RNA that impede egress from the nucleus and reduce the efficiency of translation. Identifying these elements has proven difficult, as they appear to be disseminated throughout the viral genome. RESULTS. Here, we report that **selective codon** usage appears to account for a substantial fraction of the inefficiency of viral protein synthesis, independent of any effect on improved nuclear export. The codon usage effect is not specific to transcripts of HIV-1 origin. Re-engineering the coding sequence of a model protein (Thy-1) with the most prevalent HIV-1 codons significantly impairs Thy-1 expression, whereas **altering** the coding **sequence** of the jellyfish green fluorescent protein gene to conform to the favored codons of highly expressed human proteins results in a substantial increase in expression efficiency. CONCLUSIONS. Codon-usage effects are a major impediment to the efficient expression of HIV-1 genes. Although mammalian genes do not show as profound a bias as do Escherichia coli genes, other proteins that are poorly expressed in mammalian cells can benefit from codon re-engineering.

L109 ANSWER 42 OF 105 LIFESCI COPYRIGHT 2004 CSA on STN DUPLICATE 16
AB The cloning and expression of Bacillus thuringiensis delta -endotoxin genes in transgenic plants have been used with the objective of protecting the crops from insect attack. The increased expression of the insecticidal cry genes in plants has been critical for the development of genetically transformed plants with agronomically acceptable levels of insect resistance. Low expression levels of such genes also have an environmental implication: the release of low expressing insect-tolerant transgenic plants may result in the rapid appearance of resistance to the Cry toxin

in the target insect. The problem of the expression of B. thuringiensis cry genes is due to the expression of bacterial prokaryotic genes in higher plants or in any other eukaryotic organism. Fully **modified genes** can express up to 100-fold higher levels of the insecticidal toxin compared to those obtained when a wild-type bacterial gene is expressed. We describe the most important aspects present in the bacterial wild-type cry genes affecting their expression in transgenic plants. The analysis includes aspects of transcriptional regulation, mRNA stability, **preferences** in **codon** usage and translational efficiency. According to these considerations, **modified** cry **genes** have been reconstructed allowing to increase the expression levels in transgenic plants.

L109 ANSWER 44 OF 105 MEDLINE on STN DUPLICATE 18

AB Considering the factors which affect gene transcription, translation and the stability of mRNA, without changing the amino acid composition of the encoded polypeptide, AaIT gene encoding insect-specific neurotoxin was designed and synthesized according to bias in **codon choice**, overall G+C content and G+C content of bases at the third position in codons of polyhedrin genes of baculovirus and of plant genes as well. AaIT **gene** was fused behind a **synthetic** gp67 signal **sequence** and then recombined into the genome of Trichoplusia ni nuclear polyhedrosis virus (TnNPV) by transfer vector pSXIV VI+X3. The recombinant virus TnNPV-AaIT (occ+-gal-) was screened. The results of Southern blotting and SDS-PAGE demonstrated that AaIT gene had integrated into the genome of virus and expressed. Bioassays on the 3rd-instar Trichoplusia ni larvae showed that recombinant viruses TnNPV-AaIT could shorten the time of killing insect and improve the efficacy of killing agronomically important insects.

L109 ANSWER 46 OF 105 MEDLINE on STN DUPLICATE 20

AB Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a well-known target for pyrimethamine and cycloguanil. The low amounts of enzyme obtainable from parasites or the currently available heterologous expression systems have thus far hindered studies of this enzyme. The 1912-base pair P. falciparum DHFR-TS gene was designed based on E. coli **codon preference** with unique restriction sites evenly placed throughout the coding sequence. The gene was designed and synthesized as three separated domains: the DHFR domain, the junctional sequence, and the TS domain. Each of these domains contained numerous unique restriction sites to facilitate mutagenesis. The three domains were assembled into a complete DHFR-TS gene which contained 30 unique restriction sites in the coding sequence. The bifunctional DHFR-TS was expressed from the **synthetic gene** as soluble enzyme in E. coli about 10-fold more efficiently than from the wild-type sequence. The DHFR-TS from the **synthetic gene** had kinetic properties similar to those of the wild-type enzyme and represents a convenient source of protein for further study. The unique restriction sites in the coding sequence permits easy mutagenesis of the gene which should facilitate further understanding of the molecular basis of antifolate resistance in malaria.

L109 ANSWER 47 OF 105 MEDLINE on STN DUPLICATE 21

AB A synthetic wheat high-molecular-weight (HMW) glutenin storage protein gene analog was constructed for expression in E. coli. This first **synthetic** HMW-glutenin **gene** and future **modifications** are intended to allow systematic dissection of the molecular basis of HMW-glutenin role in the visco-elastic properties critical for wheat product processing and utilization. The design of the gene included four features: different construction strategies for the separate assembly of major polypeptide domains, the inclusion of convenient restriction sites for modifications, use of a **codon selection** similar to E. coli highly expressed genes, and the ability to produce repetitive sequence domains of exact numbers of defined

repeats. The complete synthetic HMW-glutenin construct was 1908 bp, and contained 32 identical copies of one of the HMW-glutenin repetitive domain motifs. The gene expressed the novel HMW-glutenin protein to relatively high levels in bacterial cultures and the protein exhibited the known anomalous behavior of HMW-glutenins in SDS-PAGE.

L109 ANSWER 49 OF 105 MEDLINE on STN DUPLICATE 22
AB **Synthetic genes** encoding recombinant spider silk proteins have been constructed, cloned, and expressed. Protein sequences were derived from Nephila clavipes dragline silk proteins and reverse-translated to the corresponding DNA sequences. **Codon selection** was chosen to maximize expression levels in Escherichia coli. DNA "monomer" sequences were multimerized to encode high molecular weight synthetic spider silks using a "head-to-tail" construction strategy. Multimers were cloned into a prokaryotic expression vector and the encoded silk proteins were expressed in E. coli upon induction with IPTG. Four multimer, ranging in size from 14.7 to 41.3 kDa, were chosen for detailed analysis. These proteins were isolated by immobilized metal affinity chromatography and purified using reverse-phase HPLC. The composition and identity of the purified proteins were confirmed by amino acid composition analysis, N-terminal sequencing, laser desorption mass spectroscopy, and Western analysis using antibodies reactive to native spider dragline silk. Circular dichroism measurements indicate that the synthetic spider silks have substantial beta-sheet structure.

L109 ANSWER 54 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB Efficient heterologous gene expression during high cell density culture of Escherichia coli is critical to the successful commercial application of biotechnology. Various approaches to enhance gene expression, including appropriate promoter **selection**, optimized **codon** usage, ribosome enhancement, host cell genetics **modification**, **gene** dosage effect, plasmid stability, mRNA stability enhancement, host cell genetics modification and amino acid misincorporation control, are discussed in the context of improvement of both recombinant product quality and yield. These approaches will then allow successful fermentation process scale-up. (0 ref)

L109 ANSWER 56 OF 105 MEDLINE on STN DUPLICATE 27
AB I present evidence that natural **selection** biases synonymous **codon** usage to enhance the accuracy of protein synthesis in Drosophila melanogaster. Since the fitness cost of a translational misincorporation will depend on how the amino acid substitution affects protein function, selection for translational accuracy predicts an association between codon usage in DNA and functional constraint at the protein level. The frequency of preferred codons is significantly higher at codons conserved for amino acids than at nonconserved codons in 38 genes compared between D. melanogaster and Drosophila virilis or Drosophila pseudoobscura ($Z = 5.93$, $P < 10(-6)$). Preferred codon usage is also significantly higher in putative zinc-finger and homeodomain regions than in the rest of 28 D. melanogaster transcription factor encoding genes ($Z = 8.38$, $P < 10(-6)$). Mutational **alternatives** (within-**gene** differences in mutation rates, amino acid changes altering **codon preference** states, and doublet mutations at adjacent bases) do not appear to explain this association between synonymous codon usage and amino acid constraint.

L109 ANSWER 57 OF 105 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AB RAP30 and RAP74 are subunits of RAP30/74 (TFIIF, beta gamma), a general initiation and elongation factor for transcription by RNA polymerase II. Methods were previously published for production of human RAP30 and RAP74 in bacterial cells, using a bacteriophage T7 promoter expression system. The vectors described for production of RAP74 were not very efficient and produced significant quantities of RAP74 amino terminal fragments. To

improve these vectors, a segment of the human RAP74 cDNA was recoded using a preferred set of codons for translation in Escherichia coil. Recoding dramatically improved protein production and suppressed production of amino-terminal fragments. Improved vectors are reported that produce RAP74 with an LEHHHHHH carboxy-terminal extension (RAP74-H-6), for purification on a Ni2+-affinity column, and also with the native carboxy terminus (RAP74). Methods for purification of RAP74-H-6 and RAP74 are reported. Using these improved vectors, approximately 30 mg of soluble and active RAP74-H-6 or RAP74 can be produced and purified from 1 liter off. coil culture, representing a 10-fold improvement in protein production. Methods have also been developed for reconstitution of native RAP30/74 complex using recombinant proteins. This complex has indistinguishable activity from human RAP30/74 for accurate transcription in vitro. (C) 1994 Academic Press, Inc.

L109 ANSWER 66 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB A new method for determining the relative native **codon** pairing **preference** in an organism involves: obtaining nucleotide sequence data; determining the codon usage; determining the expected number of occurrences of randomly-paired codons; comparing the expected number with the actual number to determine the relative codon usage; eliminating any amino acid pair bias; and **altering** a **gene** for expression in the organism, by substituting codons according to the preferred usage, to **alter** the translational kinetics for a **gene** in a predetermined manner. The information may be used for: artificial **gene** construction, e.g. for **altering** a **gene** from a 1st organism for high-level expression in a 2nd organism; to determine the type of organism from which a sample of nucleic acid originates, by comparing the **codon** pair **preference** with standard data; and introducing a translational pause site into a gene, by introduction of an over-represented codon pair. The identification of **codon** pair **preferences** in organisms allows enhanced recombinant protein expression, controlled protein folding and evolutionary studies. (106pp)

L109 ANSWER 67 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB A computer program, which runs on MS-DOS personal computers, is described that assists in the design of **synthetic genes** coding for proteins. The goal of the program is the design of a gene which (i) contains as many unique restriction sites as possible and (ii) uses a specific codon usage. The gene designed according to the criteria above is (i) suitable for modular mutagenesis expts. and (ii) optimized for expression. The program reverse-translates protein sequences into degenerated DNA sequences, generates a map of potential restriction sites and locates sequence positions where unique restriction sites can be accommodated. The nucleic acid sequence is then refined according to a specific codon usage to remove any degeneration. Unique restriction sites, if potentially present, can be forced into the degenerated nucleic acid sequence by using priority codes assigned to different restriction sequences.

L109 ANSWER 68 OF 105 MEDLINE on STN DUPLICATE 33
AB High levels of active HIV-1 protease (PR) were produced in Escherichia coli, amounting to 8-10% of total cell protein. High production levels were achieved by altering the following parameters: (1) **codon** **preference** of the coding region, (2) A+T-richness at the 5' end of the coding region, and (3) promoter. To circumvent the toxicity of HIV-1 PR in E. coli, the gene was expressed as a fusion protein with two different proteolytic autocleavage sequences. In both the cases, the fusion protein could be cleaved in vivo to give an active molecule with the native sequence at the N terminus.

L109 ANSWER 74 OF 105 MEDLINE on STN DUPLICATE 36
AB A computer program (PINCERS) is described for use in the design of

synthetic genes and mixed-probe DNA **sequences.**
A protein sequence is reverse translated with generation of synonymous codons at each position producing a degenerate sequence. In order to locate potential restriction enzyme sites, the degenerate sequence is searched with a library of restriction enzymes for sites that utilize any combination of synonymous codons. These sites are indicated in a map so that they may be incorporated into the **synthetic gene sequence.** The program allows the user to **select** the appropriate **codon** usage table for the organism of interest and then to set a threshold usage frequency below which codons are not generated. PINCERS may also be used to assist in planning the synthesis of mixed-probe DNA sequences for cross-hybridization experiments. It can identify regions of specified length with the protein sequence that have the least overall degeneracy, thereby minimizing the number of probes to be synthesized and, therefore, maximizing the concentration of a given probe sequence.

L109 ANSWER 78 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB Bovine insulin-like growth factor 2 (bIGF2) was produced in inclusion bodies in the cytoplasm of E. coli and accumulated at high levels: 20-25% of total Coomassie-stained bacterial protein. The level of accumulation of bIGF2 was affected by the choice of codons in the 5' end of the coding sequence and by a rpoH mutation in the host cells. Purified recombinant bIGF2 had the native N terminus and the same mitogenic activity as that of bIGF2 purified from bovine serum.

L109 ANSWER 80 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB A computer aided methodology was provided for the generation of artificial genes. This strategy allowed a complete control over factors such as **codon** usage, **selection** for efficient controllable promoters and initiation signals, and enabled elimination of sequences which interfere with efficient transcription or translation (e.g. secondary structures in RNA). Provision of convenient restriction sites for gene manipulation including site-directed mutagenesis for protein engineering were important assets of the technique. The validity of this method was demonstrated by the construction of the human interleukin-2 (IL-2) gene and 3 of its mutants. The artificial genes were expressed in Escherichia coli MC1060 under the control of the E. coli trp promoter/operator. IL-2 expression levels at the stationary phase of bacterial growth were up to 11-15% of total bacterial proteins. The recombinant IL-2 protein precipitated within the bacterial cells as inclusion bodies. IL-2 and its 3 analogs were active in vitro. Similar approaches can be adopted for any other protein of known primary sequence. (14 ref)

L109 ANSWER 92 OF 105 MEDLINE on STN DUPLICATE 44
AB A DNA duplex coding for the 27 amino acids of secretin has been synthesized and cloned. In designing the sequence of the gene, computer analysis has been applied. The following factors have been considered: **selection** of **codon** usage in favour of expression in yeast; design of various sites useful in **gene** cloning, **gene modification** and expressed product purification; avoiding the repeat sequences which may interfere in the ligation of the synthetic fragments. The synthesis involved preparation of 12 oligodeoxyribonucleotides (12-mer to 24-mer in length) by phosphate triester and phosphite triester method, purification by polyacrylamide gel electrophoresis (PAGE). A new plasmid pWS1 was constructed by insertion of the enzymatic ligated gene fragment into plasmid pWR13.

L109 ANSWER 96 OF 105 MEDLINE on STN DUPLICATE 47
AB A region of minimal **codon** degeneracy was **selected** from the amino acid sequence of the amino-terminal alpha I domain of human erythrocyte spectrin to design a 90-base-pair DNA probe for the screening of a human genomic library. Five complementary oligonucleotides were

assembled to form a full-length double-stranded DNA, which was then cloned in an M13 phage vector to generate hybridization probes. Under stringent conditions, a single hybridizing clone was isolated from a total human genomic library. Partial DNA sequence analysis established the 16.8-kilobase-pair isolate as erythrocyte alpha-spectrin by correlation to a known sequence of 131 amino acids. The spectrin 106 amino acid repeat segment is encoded by multiple exons separated by introns of various sizes. Of the 3074 base pairs of DNA sequenced thus far, 12.8% code for amino acids.

L109 ANSWER 98 OF 105 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
AB Through the use of recently developed recombinant DNA methods, high level expression systems for the production of recombinant proteins such as interferons in microorganisms now exist. Using a defined medium and a multiple-phage resistant AM-7 strain of Escherichia coli containing a temperature-sensitive multicopy plasmid under lambda promoter control, a fermentation process has been developed for an analog human interferon-alpha (IFN-alpha) which is a 'consensus' of the known IFN-alpha subtypes (IFN-alpha-Con1). The **gene** for the interferon was **synthetic** and **codon choices** were optimized for expression in E.coli. Cultures were performed at 30 deg in a chemostat, at pH 7 with shaking. Fed-batch culture was also performed. A culture medium containing glucose was used. Yields of up to 7.6 x 10 power 12 U IFN-alpha-Con1/l of fermentation broth were obtained. The combination of a tightly regulated expression system and a controlled feeding schedule was required for the high expression levels and cell densities necessary to give this yield. (20 ref)

L109 ANSWER 100 OF 105 MEDLINE on STN DUPLICATE 49
AB A **synthetic** human growth hormone (hGH) **gene** was efficiently expressed under the control of the repressible acid phosphatase promoter in yeast (Saccharomyces cerevisiae). More than 10(6) molecules of hormone were formed per cell despite the fact that the gene was constructed with **codon preference** for Escherichia coli.

L109 ANSWER 103 OF 105 HCAPLUS COPYRIGHT 2004 ACS on STN
AB A rapid, efficient procedure for the total (chemical and enzymic) synthesis of linear, double-stranded DNA sequences of up to .apprx.200 base pairs is described. The method involves the chemical synthesis of oligodeoxyribonucleotides with regions of base complementarity, the annealing of the oligonucleotides, and the joining of oligonucleotides by enzymic methods. The **codon preference** of Escherichia coli is considered in the synthesis, and the redundancy of the genetic code is exploited to reduce self-complementarity in the oligonucleotides synthesized. The method is employed in the synthesis and cloning of genes for human β-endorphin [61214-51-5] and leucine-5 human β-endorphin [66238-14-0]. Thus, oligodeoxyribonucleotides were chemical synthesized by the described method, which involved a solid support; the oligonucleotides were joined to form genes for human β-endorphin and a leucine-5 derivative The fusion of genes for β-endorphin and derivs. to genes for β-galactosidase [9031-11-2] or β-lactamase [9073-60-3] was described, as was the cloning of **synthetic genes** in E. coli. The preparation of 125I-labeled **synthetic genes** and antibodies to **gene** products was claimed.

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